USE OF GLP1- AGONISTS IN THE TREATMENT OF PATIENTS WITH TYPE I DIABETES

FIELD OF THE INVENTION

The present invention relates to the use of GLP-1 agonists in the treatment of patients with type I diabetes, where the patient is newly diagnosed with type 1 diabetes when treatment with the GLP-1 agonist is initiated. The present invention also relates to the use of GLP-1 agonists to prolong the remission period in such patients. The present invention further relates to a diagnostic test which utilizes measurement of endogenous GLP-1 levels to predict loss of beta cell function in patients with type I diabetes. The present invention further relates to methods for determining whether a patient with type 1 diabetes is in need of treatment with a GLP-1 agonist and/or is in remission.

BACKGROUND OF THE INVENTION

Type 1 diabetes is characterised by a progressive loss of pancreatic beta cells due to an unfavourable balance between the destructive autoimmune processes targeting beta cells on the one hand and the regenerative capacity of these cells on the other hand. This imbalance eventually leads to total loss of beta cells and endogenous insulin secretion. However, shortly after insulin treatment is initiated in newly diagnosed patients with type 1 diabetes a paradoxical improvement occurs: the patient's residual beta-cell capacity increases and a remission period ("honeymoon") follows in which the patient's need for exogenous insulin treatment is lowered and in some cases even totally abolished and metabolic control is near to optimal [Büyükgebiz A et al Journal of Pediatric Endocrinology & Metabolism (2001);114: 1585-1596]. Consequently the remission period may reflect a period of relative beta-cell recovery or the clinical presentation of an injured but still regenerating beta cell mass. Accordingly, the duration of the remission period will be proportional to the regeneration potential of the beta cells [Larsson L-I. Microsc Res Tech (1998) 43: 284-291; Picket R, et al Dev Biol (1972) 29: 436-467, and Bonner-Weir S, et al Diabetes (1993) 42(12):1715-20]. It is therefore possible that an increase in the beta cell mass in newly diagnosed patients with type 1 diabetes may relieve the stress on the remaining beta cells and thus protect them against autoimmune destruction.

The principle of pharmacological intervention to preserve beta-cell function in the remission period has previously been demonstrated with diazoxide as an adjunct therapy to regular insulin regimen in islet cell antibody (ICA)- positive adults patients with newly diagnosed type 1 diabetes [Björk E, et al Diabetes (1996) 45:1427-30 and Björk E, et al Diabetes Care (1998) 21:427-430) but due to intolerable side effects (lowering of blood pressure,

edema, increased hair growth) treatment with this compound is not amendable to normal clinical praxis. Therefore at present there is no basis for pharmacological intervention to preserve beta-cell function in the remission period on a regular basis.

Human GLP-1 is a 37 amino acid residue peptide originating from preproglucagon which is synthesized i.a. in the L-cells in the distal ileum, in the pancreas and in the brain. GLP-1 is an important gut hormone with regulatory function in glucose metabolism and gastrointestinal secretion and metabolism. Processing of preproglucagon to give GLP-1(7-36)amide, GLP-1(7-37) and GLP-2 occurs mainly in the L-cells. A simple system is used to describe fragments and analogues of this peptide. Thus, for example, Gly^{8}-GLP-1(7-37) designates a fragment of GLP-1 formally derived from GLP-1 by deleting the amino acid residues Nos. 1 to 6 and substituting the naturally occurring amino acid residue in position 8 (Ala) by Gly. Similarly, $Lys^{34}(N^{\varepsilon}$-tetradecanoyl)-GLP-1(7-37) designates GLP-1(7-37) wherein the ε-amino group of the Lys residue in position 34 has been tetradecanoylated.

In type 2 diabetes it is known that patients have impaired secretion of GLP-1 during a meal tolerance test, an impairment that may account for the disturbances in insulin and glucagon secretion seen in that disease, and the use of GLP-1 in the treatment of type 2 diabetics has been widely described in patents and in the literature.

WO 95/31214 describes the treatment of type 1 diabetics with insulin and a GLP-1 related peptide.

SUMMARY OF THE INVENTION

The present invention relates to a method for treating a patient with type 1 diabetes, said method comprising administering to said patient an effective amount of a GLP-1 agonist or a pharmaceutically acceptable salt thereof, where the patient is newly diagnosed with type 1 diabetes when the GLP-1 agonist is first administered to the patient.

In one embodiment of the aforementioned method of treating a patient with type 1 diabetes, the patient is further administered insulin.

In another embodiment of the aforementioned method of treating a patient with type 1 diabetes, the patient is further administered an autoimmune agent.

The present invention also relates to a method for prolonging the time a patient with type 1 diabetes is in remission, said method comprising administering to a type 1 diabetes patient in remission an amount of GLP-1 agonist effective to prolong the time said patient is in remission, where the patient is newly diagnosed with type 1 diabetes when the GLP-1 agonist is first administered to the patient.

In one embodiment of the aforementioned method for prolonging the time a patient with type 1 diabetes is in remission, the patient is further administered an autoimmune agent.

The present invention further relates to a method for predicting whether a patient with type I diabetes will suffer a decrease in beta cell function, said method comprising analyzing a sample from said patient to determine the concentration of endogenous GLP-1 (7-37) and GLP-1 (7-36) amide in said sample, where the greater the concentration of endogenous GLP-1 (7-37) and GLP-1 (7-36) amide in said sample, the greater the risk that said patient will suffer a decrease in beta cell function.

The present invention also relates to a method for determining whether to administer a GLP-1 agonist to a patient with type 1 diabetes, said method comprising analyzing a sample from said patient to determine the concentration of endogenous GLP-1 (7-37) and GLP-1 (7-36) amide in said sample, where a concentration of endogenous GLP-1 (7-37) and GLP-1 (7-36) amide of greater than 25 pmol/l in said sample indicates that said patient should be administered a GLP-1 agonist.

The present invention also relates to a method for determining whether to administer a GLP-1 agonist to a patient with type 1 diabetes, said method comprising calculating the sum of said patient's HbA_{1c} level on a given day and four times the patient's daily insulin dose for said day, where a sum of less than 9% indicates that said patient should be administered a GLP-1 agonist.

The present invention further relates to a method for determining whether a patient with type I diabetes is in remission, said method comprising calculating the sum of said patient's HbA_{1c} level on a given day and four times the patient's daily insulin dose for said day, where a sum of less than 9% indicates that said patient is in remission.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a correlation between C peptide and endogenous GLP-1 levels [ie GLP-1 (7-37) and GLP-1 (7-36) amide] in 276 children one month after diagnosis with type 1 diabetes.

Figure 2 shows a correlation between C peptide and endogenous GLP-1 levels [ie GLP-1 (7-37) and GLP-1 (7-36) amide] in 276 children six months after diagnosis with type 1 diabetes.

Figure 3 shows a correlation between C peptide and endogenous GLP-1 levels [ie GLP-1 (7-37) and GLP-1 (7-36) amide] in 276 children twelve months after diagnosis with type 1 diabetes.

Figure 4 shows a correlation between HbA_{1c} and endogenous GLP-1 levels [ie GLP-1 (7-37) and GLP-1 (7-36) amide] in 276 children one month after diagnosis with type 1 diabetes.

Figure 5 shows a correlation between HbA_{1c} and endogenous GLP-1 levels [ie GLP-1 (7-37) and GLP-1 (7-36) amide] in 276 children three months after diagnosis with type 1 diabetes.

Figure 6 shows a correlation between HbA_{1c} and endogenous GLP-1 levels [ie GLP-1 (7-37) and GLP-1 (7-36) amide] in 276 children twelve months after diagnosis with type 1 diabetes.

DESCRIPTION OF THE INVENTION

The present invention relates to a method for treating a patient with type I diabetes, said method comprising administering to said patient an effective amount of a GLP-1 agonist or a pharmaceutically acceptable salt thereof, where the patient is newly diagnosed with type 1 diabetes when the GLP-1 agonist is first administered to the patient.

By "newly diagnosed with type 1 diabetes" as used in the present application is meant that the patient has been diagnosed with type 1 diabetes within the last 12 months, preferably within the last 6 months, more preferably within the last 3 months, even more preferably within the last 2 months, and most preferably within the last month.

Of course, one skilled in the art would recognize that type 1 diabetes can be diagnosed by one or more of the following tests, including but not limited to, a urinalysis showing the presence of glucose and ketone bodies in the urine, a fasting blood glucose of 126 mg/dl or higher, a random glucose of greater than 200 mg/dl, an HbA_{1c} of greater than 6% (where the % is a % of total hemoglobin), a serum insulin test where the fasting insulin is greater than 20mcU/ml, or a C-peptide test of greater than 100 pmol/l.

In one embodiment of the methods of the invention, the patient is newly diagnosed with type 1 diabetes before 18 years of age.

In another embodiment of the methods of the invention, the patient is newly diagnosed with type 1 diabetes before 16 years of age.

In a further embodiment, the patient is newly diagnosed with type 1 diabetes while the patient is prepubescent.

In yet another embodiment, is newly diagnosed with type 1 diabetes before 12 years of age.

In a further embodiment, the patient is newly diagnosed with type 1 diabetes before 6 years of age.

In yet another embodiment, the first dose of GLP-1 agonist is administered to the patient within less than 3 months after the patient has been administered his/her first dose of insulin, more preferably within less than 2 months after the patient has been administered his/her first dose of insulin, and most preferably within less than 1 months after the patient has been administered his/her first dose of insulin.

Alternatively, the first dose of GLP-1 agonist is to be administered to said patient once the patient exhibits stable blood glucose levels for a period of at time after initiation of insulin treatment, where "stable blood glucose levels" are indicated by repeated fasting blood glucose values < 10 mmol/l (180 mg/dl). In a preferred embodiment, the period of time over which the blood glucose levels remain stable is about 14 days.

In a further embodiment of the invention, the patient will continue to be administered a GLP-1 agonist for as long as the patient's C peptide level is > 100 pmol/l, preferably >300 pmol/l. C peptide levels can be measured by methods known to those skilled in the art such as enzyme-linked immunosorbent assay (DAKO, Ely, UK).

In yet another embodiment, the GLP-1 agonist is administered to the patient for more than 4 weeks, more preferably for more than 3 months, even more preferably for more than 6 months, and most preferably, for at least 12 months.

In a further embodiment, the patient to be treated is in remission where remission may be defined in a number of ways. For example, remission may be defined as an insulin requirement of < 0.5U/kg/24h, or as an insulin requirement of < 0.5 U/kg/24h in combination with HbA_{1c} below 7.5 %, or as a basal C-peptide level of >100 pmol/l. In a preferred embodiment, remission is defined by the formula: HbA_{1c} + (4 x the daily insulin dose (U/Kg/24h) < 9%. Where the patient to be treated with the GLP-1 agonist is in remission, it is believed that treatment of the patient with the GLP-1 agonist will prolong the period said patient is in remission ("the remission period") relative to treatment in the absence of the GLP-1 agonist. Accordingly, the present invention also relates to a method for prolonging the time a patient with type I diabetes is in remission, said method comprising administering to a type 1 diabetes patient in remission an amount of a GLP-1 agonist effective to prolong the time said patient is in remission, where said remission is measured by one of the formulas described above.

In another embodiment of the methods of the invention, the patient is further administered insulin where "insulin" is understood to mean human insulin, [where "human insulin" means insulin having the amino acid sequence shown in DSHW Nicol and LF Smith: Nature, (1960) 4736:483-485, which is hereby incorporated by reference], human insulin analogs, human insulin derivatives or mixtures thereof, where examples of insulin analogs and derivatives are those disclosed in EP 0 792 290 (Novo Nordisk A/S), eg $N^{\varepsilon B29}$-tetradecanoyl des

(B30) human insulin, EP 0 214 826 and EP 0 705 275 (Novo Nordisk A/S), eg Asp^{B28} human insulin, US 5,504,188 (Eli Lilly), eg Lys^{B28} Pro^{B29} human insulin, EP 0 368 187 (Aventis), eg insulin glargine.

In the methods of the present invention, "co-administering" or "coadministration" is understood to mean separate administration of two or more medicaments each containing one active ingredient (eg insulin, a GLP-1 agonist or an autoimmune agent) at the same or different times as well as simultaneous administration of the active ingredients, whether or not the two or more active ingredients are combined in one formulation or whether they are in separate formulations. By way of example, if the patient with type 1 diabetes is to be treated with insulin and a GLP-1 agonist, the insulin and GLP-1 agonist may be administered as separate medicaments at the same or different times or, the insulin and GLP-1 agonist may be administered simultaneously as separate medicaments or as a single medicament. Further by way of example, if the patient is to be treated with insulin and a GLP-1 agonist and an autoimmune agent, the insulin and a GLP-1 agonist and an autoimmune agent may each be administered as a separate medicament at the same or different times or, the insulin and the GLP-1 agonist and the autoimmune agent may be administered simultaneously as separate medicaments or as a single medicament (ie a three in one medicament), or two of the active ingredients may be administered as a single medicament and the third active ingredient as a separate medicament, at either the same or different times;

"effective amount" or an "amount...effective" is understood to mean a dosage which is sufficient in order for the treatment of the patient with newly diagnosed type 1 diabetes to be effective compared to treatment without the administered dosage. It is to be understood that "an effective amount" is the effective dose to be determined by a qualified practitioner, who may titrate dosages to achieve the desired response. Factors for consideration of dose will include potency, bioavailability, desired pharmacokinetic/pharmacodynamic profiles, patient-related factors (e.g. weight, health, age, etc.), presence of co-administered medications, time of administration, or other factors known to a medical practitioner; and

"a GLP-1 agonist" is understood to refer to any compound, including peptides and non-peptide compounds, which fully or partially activates the human GLP-1 receptor. In a preferred embodiment, the "GLP-1 agonist" is any peptide or non-peptide small molecule that binds to a GLP-1 receptor, preferably with an affinity constant (K_D) or a potency (EC_{50}) of below 1 μM, e.g. below 100 nM as measured by methods known in the art (see e.g. WO 98/08871) and exhibits insulinotropic activity, where insulinotropic activity may be measured in vivo or in vitro assays known to those of ordinary skill in the art. For example, the GLP-1 agonist may be administered to an animal and the insulin concentration measured over time.

In one embodiment, the GLP-1 agonist is selected from the group consisting of GLP-1(7-36)-amide, GLP-1(7-37), a GLP-1(7-36)-amide analogue, a GLP-1(7-37) analogue, or a derivative of any of these.

In the present application, the designation "an analogue" is used to designate a peptide wherein one or more amino acid residues of the parent peptide have been substituted by another amino acid residue and/or wherein one or more amino acid residues of the parent peptide have been deleted and/or wherein one or more amino acid residues have been added to the parent peptide. Such addition can take place either at the N-terminal end or at the C-terminal end of the parent peptide or both. Typically " an analogue" is a peptide wherein 6 or less amino acids have been substituted and/or added and/or deleted from the parent peptide, more preferably a peptide wherein 3 or less amino acids have been substituted and/or added and/or deleted from the parent peptide, and most preferably, a peptide wherein one amino acid has been substituted and/or added and/or deleted from the parent peptide.

In the present application, "a derivative" is used to designate a peptide or analogue thereof which is chemically modified by introducing e.g. ester, alkyl or lipophilic functionalities on one or more amino acid residues of the peptide or analogue thereof. Methods for identifying GLP-1 agonists are described in WO 93/19175 (Novo Nordisk A/S) and examples of suitable GLP-1 analogues and derivatives which can be used according to the present invention includes those referred to in WO 99/43705 (Novo Nordisk A/S), WO 99/43706 (Novo Nordisk A/S), WO 99/43707 (Novo Nordisk A/S), WO 98/08871 (Novo Nordisk A/S), WO 99/43708 (Novo Nordisk A/S), WO 99/43341 (Novo Nordisk A/S), WO 87/06941 (The General Hospital Corporation), WO 90/11296 (The General Hospital Corporation), WO 91/11457 (Buckley et al.), WO 98/43658 (Eli Lilly & Co.), EP 0708179-A2 (Eli Lilly & Co.), EP 0699686-A2 (Eli Lilly & Co.), WO 01/98331 (Eli Lilly & Co).

In one embodiment, the GLP-1 agonist is a derivative of GLP-1(7-36)-amide, GLP-1(7-37), a GLP-1(7-36)-amide analogue or a GLP-1(7-37) analogue, which comprises a lipophilic substituent.

In this embodiment of the invention, the GLP-1 derivative preferably has three lipophilic substituents, more preferably two lipophilic substituents, and most preferably one lipophilic substituent attached to the parent peptide (ie GLP-1(7-36)-amide, GLP-1(7-37), a GLP-1(7-36)-amide analogue or a GLP-1(7-37) analogue), where each lipophilic substituent(s) preferably has 4-40 carbon atoms, more preferably 8-30 carbon atoms, even more preferably 8-25 carbon atoms, even more preferably 12-25 carbon atoms, and most preferably 14-18 carbon atoms.

In one embodiment, the lipophilic substituent comprises a partially or completely hydrogenated cyclopentanophenathrene skeleton.

In another embodiment, the lipophilic substituent is a straight-chain or branched alkyl group.

In yet another embodiment, the lipophilic substituent is an acyl group of a straight-chain or branched fatty acid. Preferably, the lipophilic substituent is an acyl group having the formula $CH_3(CH_2)_nCO$-, wherein n is an integer from 4 to 38, preferably an integer from 12 to 38, and most preferably is $CH_3(CH_2)_{12}CO$-, $CH_3(CH_2)_{14}CO$-, $CH_3(CH_2)_{16}CO$-, $CH_3(CH_2)_{18}CO$-, $CH_3(CH_2)_{20}CO$- and $CH_3(CH_2)_{22}CO$-. In a more preferred embodiment, the lipophilic substituent is tetradecanoyl. In a most preferred embodiment, the lipophilic substituent is hexadecanoyl.

In a further embodiment of the present invention, the lipophilic substituent has a group which is negatively charged such as a carboxylic acid group. For example, the lipophilic substituent may be an acyl group of a straight-chain or branched alkane α,ω-dicarboxylic acid of the formula $HOOC(CH_2)_mCO$-, wherein m is an integer from 4 to 38, preferably an integer from 12 to 38, and most preferably is $HOOC(CH_2)_{14}CO$-, $HOOC(CH_2)_{16}CO$-, $HOOC(CH_2)_{18}CO$-, $HOOC(CH_2)_{20}CO$- or $HOOC(CH_2)_{22}CO$-.

In the GLP-1 derivatives of the invention, the lipophilic substituent(s) contain a functional group which can be attached to one of the following functional groups of an amino acid of the parent GLP-1 peptide:

(a) the amino group attached to the alpha-carbon of the N-terminal amino acid,

(b) the carboxy group attached to the alpha-carbon of the C-terminal amino acid,

(c) the epsilon-amino group of any Lys residue,

(d) the carboxy group of the R group of any Asp and Glu residue,

(e) the hydroxy group of the R group of any Tyr, Ser and Thr residue,

(f) the amino group of the R group of any Trp, Asn, Gln, Arg, and His residue, or

(g) the thiol group of the R group of any Cys residue.

In one embodiment, a lipophilic substituent is attached to the carboxy group of the R group of any Asp and Glu residue.

In another embodiment, a lipophilic substituent is attached to the carboxy group attached to the alpha-carbon of the C-terminal amino acid.

In a most preferred embodiment, a lipophilic substituent is attached to the epsilon-amino group of any Lys residue.

In a preferred embodiment of the invention, the lipophilic substituent is attached to the parent GLP-1 peptide by means of a spacer. A spacer must contain at least two functional groups, one to attach to a functional group of the lipophilic substituent and the other to a functional group of the parent GLP-1 peptide.

In one embodiment, the spacer is an amino acid residue except Cys or Met, or a dipeptide such as Gly-Lys. For purposes of the present invention, the phrase "a dipeptide such as Gly-Lys" means any combination of two amino acids except Cys or Met, preferably a dipeptide wherein the C-terminal amino acid residue is Lys, His or Trp, preferably Lys, and the N-terminal amino acid residue is Ala, Arg, Asp, Asn, Gly, Glu, Gln, Ile, Leu, Val, Phe, Pro, Ser, Tyr, Thr, Lys, His and Trp. Preferably, an amino group of the parent peptide forms an amide bond with a carboxylic group of the amino acid residue or dipeptide spacer, and an amino group of the amino acid residue or dipeptide spacer forms an amide bond with a carboxyl group of the lipophilic substituent.

Preferred spacers are lysyl, glutamyl, asparagyl, glycyl, beta-alanyl and gamma-aminobutanoyl, each of which constitutes an individual embodiment. Most preferred spacers are glutamyl and beta-alanyl. When the spacer is Lys, Glu or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the amino group thereof may form an amide bond with a carboxyl group of the lipophilic substituent. When Lys is used as the spacer, a further spacer may in some instances be inserted between the ε-amino group of Lys and the lipophilic substituent. In one embodiment, such a further spacer is succinic acid which forms an amide bond with the ε-amino group of Lys and with an amino group present in the lipophilic substituent. In another embodiment such a further spacer is Glu or Asp which forms an amide bond with the ε-amino group of Lys and another amide bond with a carboxyl group present in the lipophilic substituent, that is, the lipophilic substituent is a N^{ε}-acylated lysine residue.

In another embodiment, the spacer is an unbranched alkane α,ω-dicarboxylic acid group having from 1 to 7 methylene groups, which spacer forms a bridge between an amino group of the parent peptide and an amino group of the lipophilic substituent. Preferably, the spacer is succinic acid.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula $CH_3(CH_2)_pNH\text{-}CO(CH_2)_qCO\text{-}$, wherein p is an integer from 8 to 33, preferably from 12 to 28 and q is an integer from 1 to 6, preferably 2.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula $CH_3(CH_2)_rCO\text{-}NHCH(COOH)(CH_2)_2CO\text{-}$, wherein r is an integer from 4 to 24, preferably from 10 to 24.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula $CH_3(CH_2)_sCO\text{-}NHCH((CH_2)_2COOH)CO\text{-}$, wherein s is an integer from 4 to 24, preferably from 10 to 24.

In a further embodiment, the lipophilic substituent is a group of the formula $COOH(CH_2)_tCO\text{-}$ wherein t is an integer from 6 to 24.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula -NHCH(COOH)$(CH_2)_4$NH-CO$(CH_2)_uCH_3$, wherein u is an integer from 8 to 18.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula $CH_3(CH_2)_vCO$-NH-$(CH_2)_z$-CO, wherein v is an integer from 4 to 24 and z is an integer from 1 to 6.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula -NHCH(COOH)$(CH_2)_4$NH-COCH($(CH_2)_2$COOH)NH-CO$(CH_2)_wCH_3$, wherein w is an integer from 10 to 16.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula -NHCH(COOH)$(CH_2)_4$NH-CO$(CH_2)_2$CH(COOH)NHCO$(CH_2)_xCH_3$, wherein x is zero or an integer from 1 to 22, preferably 10 to 16.

In yet another embodiment the GLP-1 agonist is Arg^{34}, Lys^{26}(N^{ε}-(γ-Glu(N^{α}-hexadecanoyl)))-GLP-1(7-37).

In yet another embodiment the GLP-1 agonist is selected from the group consisting of Gly^8-GLP-1(7-36)-amide, Gly^8-GLP-1(7-37), Val^8-GLP-1(7-36)-amide, Val^8-GLP-1(7-37), Val^8Asp^{22}-GLP-1(7-36)-amide, Val^8Asp^{22}-GLP-1(7-37) , Val^8Glu^{22}-GLP-1(7-36)-amide , Val^8Glu^{22}-GLP-1(7-37), Val^8Lys^{22}-GLP-1(7-36)-amide, Val^8Lys^{22}-GLP-1(7-37), Val^8Arg^{22}-GLP-1(7-36)-amide, Val^8Arg^{22}-GLP-1(7-37), Val^8His^{22}-GLP-1(7-36)-amide, Val^8His^{22}-GLP-1(7-37), analogues thereof and derivatives of any of these.

In yet another embodiment the GLP-1 agonist is selected from the group consisting of Arg^{26}-GLP-1(7-37); Arg^{34}-GLP-1(7-37); Lys^{36}-GLP-1(7-37); $Arg^{26,34}Lys^{36}$-GLP-1(7-37); $Arg^{26,34}$-GLP-1(7-37); $Arg^{26,34}Lys^{40}$-GLP-1(7-37); $Arg^{26}Lys^{36}$-GLP-1(7-37); $Arg^{34}Lys^{36}$-GLP-1(7-37); Val^8Arg^{22}-GLP-1(7-37); Met^8Arg^{22}-GLP-1(7-37);Gly^8His^{22}-GLP-1(7-37); Val^8His^{22}-GLP-1(7-37); Met^8His^{22}-GLP-1(7-37);His^{37}-GLP-1(7-37); Gly^8-GLP-1(7-37); Val^8-GLP-1(7-37); Met^8-GLP-1(7-37);Gly^8Asp^{22}-GLP-1(7-37); Val^8Asp^{22}-GLP-1(7-37); Met^8Asp^{22}-GLP-1(7-37);Gly^8Glu^{22}-GLP-1(7-37); Val^8Glu^{22}-GLP-1(7-37); Met^8Glu^{22}-GLP-1(7-37); Gly^8Lys^{22}-GLP-1(7-37); Val^8Lys^{22}-GLP-1(7-37); Met^8Lys^{22}-GLP-1(7-37); Gly^8Arg^{22}-GLP-1(7-37); $Val^8Lys^{22}His^{37}$-GLP-1(7-37); $Gly^8Glu^{22}His^{37}$-GLP-1(7-37); $Val^8Glu^{22}His^{37}$-GLP-1(7-37); $Met^8Glu^{22}His^{37}$-GLP-1(7-37);Gly^8Lys^{22} His^{37}-GLP-1(7-37); $Met^8Lys^{22}His^{37}$-GLP-1(7-37);$Gly^8Arg^{22}His^{37}$-GLP-1(7-37); $Val^8Arg^{22}His^{37}$-GLP-1(7-37); $Met^8Arg^{22}His^{37}$-GLP-1(7-37); $Gly^8His^{22}His^{37}$-GLP-1(7-37); $Val^8His^{22}His^{37}$-GLP-1(7-37); $Met^8His^{22}His^{37}$-GLP-1(7-37); Gly^8His^{37}-GLP-1(7-37); Val^8His^{37}-GLP-1(7-37); Met^8His^{37}-GLP-1(7-37);Gly^8Asp^{22} His^{37}-GLP-1(7-37); $Val^8Asp^{22}His^{37}$-GLP-1(7-37); $Met^8Asp^{22}His^{37}$-GLP-1(7-37); Arg^{26}-GLP-1(7-36)-amide; Arg^{34}-GLP-1(7-36)-amide; Lys^{36}-GLP-1(7-36)-amide; $Arg^{26,34}Lys^{36}$-GLP-1(7-36)-amide; $Arg^{26,34}$-GLP-1(7-36)-amide; $Arg^{26,34}Lys^{40}$-GLP-1(7-36)-amide; $Arg^{26}Lys^{36}$-GLP-1(7-36)-amide;

$Arg^{34}Lys^{36}$-GLP-1(7-36)-amide; Gly^{8}-GLP-1(7-36)-amide; Val^{8}-GLP-1(7-36)-amide; Met^{8}-GLP-1(7-36)-amide;$Gly^{8}Asp^{22}$-GLP-1(7-36)-amide; $Gly^{8}Glu^{22}His^{37}$-GLP-1(7-36)-amide; $Val^{8}Asp^{22}$-GLP-1(7-36)-amide; $Met^{8}Asp^{22}$-GLP-1(7-36)-amide;$Gly^{8}Glu^{22}$-GLP-1(7-36)-amide; $Val^{8}Glu^{22}$-GLP-1(7-36)-amide; $Met^{8}Glu^{22}$-GLP-1(7-36)-amide; $Gly^{8}Lys^{22}$-GLP-1(7-36)-amide; $Val^{8}Lys^{22}$-GLP-1(7-36)-amide; $Met^{8}Lys^{22}$-GLP-1(7-36)-amide; $Gly^{8}His^{22}His^{37}$-GLP-1(7-36)-amide; $Gly^{8}Arg^{22}$-GLP-1(7-36)-amide; $Val^{8}Arg^{22}$-GLP-1(7-36)-amide; $Met^{8}Arg^{22}$-GLP-1(7-36)-amide;$Gly^{8}His^{22}$-GLP-1(7-36)-amide; $Val^{8}His^{22}$-GLP-1(7-36)-amide; $Met^{8}His^{22}$-GLP-1(7-36)-amide;His^{37}-GLP-1(7-36)-amide; $Val^{8}Arg^{22}His^{37}$-GLP-1(7-36)-amide; $Met^{8}Arg^{22}His^{37}$-GLP-1(7-36)-amide; $Gly^{8}His^{37}$-GLP-1(7-36)-amide; $Val^{8}His^{37}$-GLP-1(7-36)-amide; $Met^{8}His^{37}$-GLP-1(7-36)-amide;$Gly^{8}Asp^{22}$ His^{37}-GLP-1(7-36)-amide; $Val^{8}Asp^{22}His^{37}$-GLP-1(7-36)-amide; $Met^{8}Asp^{22}His^{37}$-GLP-1(7-36)-amide; $Val^{8}Glu^{22}His^{37}$-GLP-1(7-36)-amide; $Met^{8}Glu^{22}His^{37}$-GLP-1(7-36)-amide;$Gly^{8}Lys^{22}$ His^{37}-GLP-1(7-36)-amide; $Val^{8}Lys^{22}His^{37}$-GLP-1(7-36)-amide; $Met^{8}Lys^{22}His^{37}$-GLP-1(7-36)-amide;$Gly^{8}Arg^{22}His^{37}$-GLP-1(7-36)-amide; $Val^{8}His^{22}His^{37}$-GLP-1(7-36)-amide; $Met^{8}His^{22}His^{37}$-GLP-1(7-36)-amide; and derivatives thereof.

In yet another embodiment the GLP-1 agonist is selected from the group consisting of $Val^{8}Trp^{19}Glu^{22}$-GLP-1(7-37), $Val^{8}Glu^{22}Val^{25}$-GLP-1(7-37), $Val^{8}Tyr^{16}Glu^{22}$-GLP-1(7-37), $Val^{8}Trp^{16}Glu^{22}$-GLP-1(7-37), $Val^{8}Leu^{16}Glu^{22}$-GLP-1(7-37), $Val^{8}Tyr^{18}Glu^{22}$-GLP-1(7-37), $Val^{8}Glu^{22}His^{37}$-GLP-1(7-37), $Val^{8}Glu^{22}Ile^{33}$-GLP-1(7-37), $Val^{8}Trp^{16}Glu^{22}Val^{25}Ile^{33}$-GLP-1(7-37), $Val^{8}Trp^{16}Glu^{22}Ile^{33}$-GLP-1(7-37), $Val^{8}Glu^{22}Val^{25}Ile^{33}$-GLP-1(7-37), $Val^{8}Trp^{16}Glu^{22}Val^{25}$-GLP-1(7-37), analogues thereof and derivatives of any of these.

In yet another embodiment the GLP-1 agonist is a stable GLP-1 analogue/-derivative. Throughout this application a "stable GLP-1 analogue/derivative" means a GLP-1 analogue or a derivative of a GLP-1 analogue which exhibits an in vivo plasma elimination half-life of at least 10 hours in man, as determined by the method described below. Examples of stable GLP-1 analogue/derivatives can be found in WO 98/08871 and WO 99/43706. The method for determination of plasma elimination half-life of a compound in man is: The compound is dissolved in an isotonic buffer, pH 7.4, PBS or any other suitable buffer. The dose is injected peripherally, preferably in the abdominal or upper thigh. Blood samples for determination of active compound are taken at frequent intervals, and for a sufficient duration to cover the terminal elimination part (e.g. Pre-dose, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 (day 2), 36 (day 2), 48 (day 3), 60 (day 3), 72 (day 4) and 84 (day 4) hours post dose). Determination of the concentration of active compound is performed as described in Wilken et al., Diabetologia 43(51):A143, 2000. Derived pharmacokinetic parameteres are calculated from the concentration-time data for each individual subject by use of non-compartmental methods, using the commercially available software WinNonlin Version 2.1 (Pharsight, Cary, NC,

USA). The terminal elimination rate constant is estimated by log-linear regression on the terminal log-linear part of the concentration-time curve, and used for calculating the elimination half-life.

Stable GLP-1 analogues and derivatives are disclosed in WO 98/08871 (analogues with lipophilic substituent) and in WO 02/46227 (analogues fused to serum albumin or to Fc portion of an Ig).

In yet another embodiment the GLP-1 agonist is exendin-4 or exendin-3 , an exendin-4 or exendin-3 analogue or a derivative of any of these.

Examples of exendins as well as analogues, derivatives, and fragments thereof to be included within the present invention are those disclosed in WO 97/46584, US 5,424,286 and WO 01/04156. US 5,424,286 describes a method for stimulating insulin release with an exendin polypeptide. The exendin polypeptides disclosed include HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGX; wherein X = P or Y, and HX1X2GTFITSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS; wherein X1X2 = SD (exendin-3) or GE (exendin-4)). WO 97/46584 describes truncated versions of exendin peptide(s). The disclosed peptides increase secretion and biosynthesis of insulin, but reduce those of glucagon. WO 01/04156 describes exendin-4 analogues and derivatives as well as the preparation of these molecules. Exendin-4 analogues stabilized by fusion to serum albumin or Fc portion of an Ig are disclosed in WO 02/46227.

In one embodiment, the exendin-4 analogue is HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPSKKKKKK.

In yet another embodiment the GLP-1 agonist is a stable exendin-4 analogue/-derivative. The term "stable exendin-4 analogue/derivative", as used herein refers to an exendin-4(1-39) analogue or a derivative of an exendin-4(1-39) analogue which exhibits an in vivo plasma elimination half-life of at least 10 hours in man, as determined by the method described above for a "stable GLP-1 analogue/derivative".

In still another embodiment the GLP-1 agonist is selected from the non-peptide small molecule GLP-1 agonists disclosed in WO 00/42026.

The present invention also encompasses pharmaceutically acceptable salts of the GLP-1 agonists. Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, ammonium and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, nitric acids and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, lactic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methane-

sulfonic, ethanesulfonic, tartaric, ascorbic, pamoic, bismethylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, EDTA, glycolic, p-aminobenzoic, glutamic, benzenesulfonic, p-toluenesulfonic acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in J. Pharm. Sci. 1977, 66, 2. Examples of metal salts include lithium, sodium, potassium, magnesium salts and the like. Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium, tetramethylammonium salts and the like.

Also intended as pharmaceutically acceptable acid addition salts are the hydrates which the present GLP-1 agonists are able to form.

Peptide GLP-1 compounds can be produced by appropriate derivatization of an appropriate peptide backbone which has been produced by recombinant DNA technology or by peptide synthesis (e.g. Merrifield-type solid phase synthesis) as known in the art of peptide synthesis and peptide chemistry.

As Type 1 diabetes is caused by an autoimmune destruction of the pancreatic beta cells, the above described methods of the present invention may further comprise administering to said patient an effective amount of an autoimmune agent where by "autoimmune agent" as used in the context of the present application is meant an agent that can inhibit or delay the autoimmune destruction of the pancreatic beta cells. As T cells play an important role in this process by mediating the autoimmune destruction, such an autoimmune agent will preferably be an agent that is capable of regulation of T cells or of the immune system's response to T cells.

Examples of "autoimmune agents" which may be utilized in the methods of the present invention include, but are not limited to, agents such as spironolactone that inhibit the release of inflammatory cytokines, immunosuppressive agents such as sirolimus, tacrolimius, and daclizumab described in Shapiro et al [N. Engl. J. Med., (2000) 343:230-238], a compound that interacts with CD3 and modulates the effects of CD3 ("a modulator of CD3"), such as an antibody reactive with CD3, where such compounds are described in PCT/DK03/00387, or glutamic acid decarboxylase (GAD) polypeptide or a peptide fragment thereof that includes any sequence of amino acids having an epitope (typically 5-12 amino acids in length) for autoantibodies to GAD or that binds to a T cell major histocompatibility ("MHC") receptor where GAD and peptide fragments thereof are described in, for example, US Patent Nos. 5,998,366 and 6,682,906.

In one embodiment, the combination of an autoimmune agent with a beta cell trophic agent such as a GLP-1 agonist is believed lengthen the time a patient with type 1 diabe-

tes is in remission relative to monotherapy with a beta cell trophic agent by acting to improve preservation of long term residual beta cell function to a greater extent than with monotherapy alone.

Of course, it would be understood by the skilled artisan that when a GLP-1 agonist is administered together with an autoimmune agent in the methods of the present invention, that the dosage of the GLP-1 agonist might be reduced relative to the dosage when the agonist is administered in the absence of the autoimmune agent.

The route of administration of GLP-1 agonists , insulin and autoimmune agents may be any route which effectively transports the active compound to the appropriate or desired site of action, such as oral, nasal, buccal, pulmonal, transdermal or parenteral.

Medicaments or pharmaceutical compositions containing a GLP-1 agonist such as Arg^{34}, $Lys^{26}(N^{\varepsilon}$-(γ-Glu(N^{α}-hexadecanoyl)))-GLP-1(7-37) or an autoimmune agent or an insulin, may be administered parenterally to a patient in need thereof. Parenteral administration may be performed by subcutaneous, intramuscular or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a powder or a liquid for the administration of a GLP-1 agonist in the form of a nasal or pulmonal spray. As a still further option, the GLP-1 agonist can also be administered transdermally, *e.g.* from a patch, optionally a iontophoretic patch, or transmucosally, *e.g.* bucally. The above-mentioned possible ways to administer GLP-1 agonists, insulin and autoimmune agents are not considered as limiting the scope of the invention.

Pharmaceutical compositions containing GLP-1 agonists such as Arg^{34}, $Lys^{26}(N^{\varepsilon}$-(γ-Glu(N^{α}-hexadecanoyl)))-GLP-1(7-37) and/or autoimmune agents, and/or insulin, may be prepared by conventional techniques, *e.g.* as described in Remington's *Pharmaceutical Sciences*, 1985 or in Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

Thus, injectable compositions of GLP-1 agonists, insulin and autoimmune agents can be prepared using the conventional techniques of the pharmaceutical industry which involves dissolving and mixing the ingredients as appropriate to give the desired end product.

For example, a GLP-1 agonist such as Arg^{34}, $Lys^{26}(N^{\varepsilon}$-(γ-Glu(N^{α}-hexadecanoyl)))-GLP-1(7-37) may be dissolved in an amount of water which is somewhat less than the final volume of the composition to be prepared. An isotonicity agent, a preservative and a buffer are added as required and the pH value of the solution is adjusted - if necessary - using an acid, *e.g.* hydrochloric acid, or a base, *e.g.* aqueous sodium hydroxide as needed. Finally, the volume of the solution is adjusted with water to give the desired concentration of the ingredients.

In one embodiment of the invention, the formulation of the GLP-1 agonist has a pH in the range from 7.0 to 10. In another embodiment of the invention the formulation has a pH in the range from 7.0 to 9.5. In a further embodiment of the invention the formulation has a pH in the range from 7.0 to 8.5. In yet another embodiment of the invention the formulation has a pH in the range from 7.0 to 8.0, preferably from 7.4 to 7.8. In a further embodiment of the invention the formulation has a pH in the range from 9.0 to 10.

Examples of isotonic agents to be used in the formulations of the invention are those selected from the group consisting of a salt (e.g. sodium chloride), a polyhydric alcohol (e.g., xylitol, mannitol, sorbitol or glycerol), a monosaccharide (e.g. glucose or maltose), a disccharide (e.g. sucrose), an amino acid (e.g. L-glycine, L-histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine), polyethyleneglycol (e.g. PEG400), or mixtures thereof. In a further embodiment of the invention the isotonic agent is selected from the group consisting of sodium chloride, glycerol, mannitol, glucose, sucrose, L-glycine, L-histidine, arginine, lysine or mixtures thereof. Each one of these specific isotonic agents constitutes an alternative embodiment of the invention.

Examples of preservatives to be used in the formulations of the invention are phenol, m-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, benzyl alcohol, chlorobutanol, and thiomerosal, or mixtures thereof. Each one of these specific preservatives constitutes an alternative embodiment of the invention. In a preferred embodiment of the invention the preservative is phenol or m-cresol.

Examples of suitable buffers to be used in the formulations of the invention are sodium acetate, sodium carbonate, citrate, glycylglycine, histidine, glycine, lysine, arginine, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate, and tris(hydroxymethyl)-aminomethan, or mixtures thereof. Each one of these specific buffers constitutes an alternative embodiment of the invention. In a preferred embodiment of the invention the buffer is glycylglycine, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate or mixtures thereof.

Further to the above-mentioned components, solutions containing a GLP-1 agonist may also contain a surfactant in order to improve the solubility and/or the stability of the peptide. In a further embodiment of the invention the formulation further comprises a surfactant. In a further embodiment of the invention the surfactant is selected from a detergent, ethoxylated castor oil, polyglycolyzed glycerides, acetylated monoglycerides, sorbitan fatty acid esters, poloxamers, such as 188 and 407, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene derivatives such as alkylated and alkoxylated derivatives

(tweens, e.g. Tween-20, or Tween-80), monoglycerides or ethoxylated derivatives thereof, diglycerides or polyoxyethylene derivatives thereof, glycerol, cholic acid or derivatives thereof, lecithins, alcohols and phospholipids, glycerophospholipids (lecithins, kephalins, phosphatidyl serine), glyceroglycolipids (galactopyransoide), sphingophospholipids (sphingomyelin), and sphingoglycolipids (ceramides, gangliosides), DSS (docusate sodium, CAS registry no [577-11-7]), docusate calcium, CAS registry no [128-49-4]), docusate potassium, CAS registry no [7491-09-0]), SDS (sodium dodecyl sulfate or sodium lauryl sulfate), dipalmitoyl phosphatidic acid, sodium caprylate, bile acids and salts thereof and glycine or taurine conjugates, ursodeoxycholic acid, sodium cholate, sodium deoxycholate, sodium taurocholate, sodium glycocholate, N-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, anionic (alkyl-aryl-sulphonates) monovalent surfactants, palmitoyl lysophosphatidyl-L-serine, lysophospholipids (e.g. 1-acyl-sn-glycero-3-phosphate esters of ethanolamine, choline, serine or threonine), alkyl, alkoxyl (alkyl ester), alkoxy (alkyl ether)-derivatives of lysophosphatidyl and phosphatidylcholines, e.g. lauroyl and myristoyl derivatives of lysophosphatidylcholine, dipalmitoylphosphatidylcholine, and modifications of the polar head group, that is cholines, ethanolamines, phosphatidic acid, serines, threonines, glycerol, inositol, and the postively charged DODAC, DOTMA, DCP, BISHOP, lysophosphatidylserine and lysophosphatidylthreonine, zwitterionic surfactants (e.g. N-alkyl-N,N-dimethylammonio-1-propanesulfonates, 3-cholamido-1-propyldimethylammonio-1-propanesulfonate, dodecylphosphocholine, myristoyl lysophosphatidylcholine, hen egg lysolecithin), cationic surfactants (quarternary ammonium bases) (e.g. cetyl-trimethylammonium bromide, cetylpyridinium chloride), non-ionic surfactants, polyethyleneoxide/polypropyleneoxide block copolymers (Pluronics/Tetronics, Triton X-100, Dodecyl β-D-glucopyranoside) or polymeric surfactants (Tween-40, Tween-80, Brij-35), fusidic acid derivatives- (e.g. sodium tauro-dihydrofusidate etc.), long-chain fatty acids and salts thereof C6-C12 (eg. oleic acid and caprylic acid), acylcarnitines and derivatives, N^{α}-acylated derivatives of lysine, arginine or histidine, or side-chain acylated derivatives of lysine or arginine, N^{α}-acylated derivatives of dipeptides comprising any combination of lysine, arginine or histidine and a neutral or acidic amino acid, N^{α}-acylated derivative of a tripeptide comprising any combination of a neutral amino acid and two charged amino acids, or the surfactant may be selected from the group of imidazoline derivatives, or mixtures thereof. Each one of these specific surfactants constitutes an alternative embodiment of the invention.

The use of isotonicity agents, preservatives, and surfactants are well knowmn in the pharmaceutical arts and reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

In a further embodiment of the invention the GLP-1 agonist is present in a formulation of the invention in a concentration from 0.1mg/ml to 80mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 1mg/ml to 80mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 0.1mg/ml to 50mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 1mg/ml to 50mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 0.1mg/ml to 20mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 1mg/ml to 20mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 0.1mg/ml to 10mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 1mg/ml to 10mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 0.1-5mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 1-5mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 0.1-0.5mg/ml. In a further embodiment of the invention the GLP-1 agonist is present in a concentration from 0.6-1mg/ml. Each one of these specific concentration ranges constitutes an alternative embodiment of the invention.

In one embodiment, the dosage of GLP-1 agonist to be administered to a patient in a method of the invention is from about 0.1 ug/kg/day to about 200ug/kg/day.

In one embodiment, the dosage of GLP-1 agonist to be administered to a patient in a method of the invention is from about 0.1 ug/kg/day to about 20ug/kg/day.

In one embodiment, the dosage of GLP-1 agonist to be administered to a patient in a method of the invention is from about 0.5 ug/kg/day to about 20 ug/kg/day.

In another embodiment, the dosage of GLP-1 agonist to be administered to a patient in a method of the invention is from about 0.5 ug/kg/day to about 2 ug/kg/day.

Of course, one skilled in the art would understood that when a GLP-1 agonist is coadministered with an autoimmune agent in the methods of the invention, the dosage of the GLP-1 agonist to be administered may be less than the dosage administered for the GLP-1 agonist in the absence of the autoimmune agent.

The dosage regimen for the autoimmune agent of the present invention will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular autoimmune agent that is to be used in the methods of the invention and its mode and route of administration; the species, age, sex, health, medical condition, and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; the route of administration, the renal and hepatic function of the patient, and the effect desired. A physician or can determine and prescribe the effec-

tive amount of the drug required to prevent, counter, or arrest the progress of the type I diabetes.

In one embodiment, where the autoimmune agent is GAD or a peptide fragment thereof, the dosage of the autoimmune agent can vary from about 0.1 mg/m^2 to about 2000 mg/m^2, preferably about 0.1 mg/m^2 to about 500 mg/m^2/dose in one or more dose administrations daily for one or more days.

The present invention also relates to a method for predicting whether a patient with type I diabetes will suffer a decrease in beta cell function, said method comprising analyzing a sample from said patient to determine the concentration of endogenous GLP-1 (7-37) and GLP-1 (7-36) amide in said sample, where the greater the concentration of endogenous GLP-1 (7-37) and GLP-1 (7-36) amide in said sample, the greater the risk that said patient will suffer a decrease in beta cell function.

By "sample" as used in the present application is meant blood, plasma or serum.

Levels of endogenous GLP-1 (7-37) and GLP-1 (7-36) amide in a sample to be determined in the methods of the invention can be obtained by methods known to those of ordinary skill in the art.

In one embodiment, the sample to be analyzed is obtained from a patient under 18 years of age.

In another embodiment, the sample to be analyzed is obtained from a patient under 16 years of age.

In a further embodiment, the sample to be analyzed is obtained from a patient who is prepubescent.

In yet another embodiment, the sample to be analyzed is obtained from a patient under 12 years of age.

In yet another embodiment, the sample to be analyzed is obtained from a patient under 6 years of age.

In a further embodiment, the sample to be analyzed is obtained from a patient who has been newly diagnosed with type 1 diabetes where by "newly diagnosed with type 1 diabetes" as used in the present application is meant that the patient has been diagnosed with type 1 diabetes within the last 12 months, preferably within the last 6 months, more preferably within the last 3 months, even more preferably within the last 2 months, and most preferably within the last month.

In yet another embodiment, the sample to be analyzed is obtained from the patient within three hours after the patient has eaten a meal, preferably within two hours after the

patient has eaten a meal and more preferably within 90 minutes after the patient has eaten a meal.

In a further embodiment, the decrease in beta cell function is determined by measuring the patient's C-peptide level in said sample ("first sample") and comparing it to the patient's C-peptide level in a second sample obtained from said patient within one year from when the first sample was obtained, more preferably within six months from when the first sample was obtained and most preferably within 2 months from when the first sample was obtained, where a reduction in C-peptide levels in the second sample relative to the first sample, indicates a decrease in beta cell function.

In one embodiment, the C peptide level is measured in a sample obtained from the patient within three hours after the patient has eaten a meal, preferably within two hours after the patient has eaten a meal and more preferably within 90 minutes after the patient has eaten a meal.

The present invention further relates to a method for determining whether to administer a GLP-1 agonist to a patient with type 1 diabetes, said method comprising analyzing a sample from said patient to determine the concentration of endogenous GLP-1 (7-37) and GLP-1 (7-36) amide in said sample, where a concentration of endogenous GLP-1 (7-37) and GLP-1 (7-36) amide of greater than 25 pmol/l in said sample indicates that said patient should be administered a GLP-1 agonist.

In one embodiment, the sample to be analyzed is from a patient under 18 years of age.

In another embodiment, the sample to be analyzed is from a patient under 16 years of age.

In yet another embodiment, the sample to be analyzed is from a patient under 12 years of age.

In a further embodiment, the sample to be analyzed is from a patient under 6 years of age.

In yet another embodiment, the sample to be analyzed is from a patient who is prepubescent.

In a further embodiment, the sample to be analyzed is obtained from a patient who has been newly diagnosed with type 1 diabetes where by "newly diagnosed with type 1 diabetes" as used in the present application is meant that the patient has been diagnosed with type 1 diabetes within the last 12 months, preferably within the last 6 months, more preferably within the last 3 months, even more preferably within the last 2 months, and most preferably within the last month.

In yet another embodiment, the sample to be analyzed is obtained from the patient within three hours after the patient has eaten a meal, preferably within two hours after the patient has eaten a meal and more preferably within 90 minutes after the patient has eaten a meal.

The present invention also relates to a method for determining whether to administer a GLP-1 agonist to a patient with type 1 diabetes, said method comprising calculating the sum of said patient's HbA_{1c} level on a given day and four times the patient's daily insulin dose for said day, where a sum of less than 9% indicates that said patient should be administered a GLP-1 agonist.

Methods for measuring a patient's HbA_{1c} levels are known to those of ordinary skill in the art and include high pressure liquid chromatography.

In one embodiment, the patient's HbA_{1c} levels are measured in a sample obtained from a patient under 18 years of age.

In another embodiment, the patient's HbA_{1c} levels are measured in a sample obtained from a patient under 16 years of age.

In a further embodiment, the patient's HbA_{1c} levels are measured in a sample obtained from a patient who is prepubescent.

In yet another embodiment, the patient's HbA_{1c} levels are measured in a sample obtained from a patient under 12 years of age.

In yet another embodiment, the patient's HbA_{1c} levels are measured in a sample obtained from a patient under 6 years of age.

In a further embodiment, the sample to be analyzed is obtained from a patient who has been newly diagnosed with type 1 diabetes where by "newly diagnosed with type 1 diabetes" as used in the present application is meant that the patient has been diagnosed with type 1 diabetes within the last 12 months, preferably within the last 6 months, more preferably within the last 3 months, even more preferably within the last 2 months, and most preferably within the last month.

Since HbA1C is a biomarker for efficacy of GLP-1, the present invention also relates to a method for determining whether to adjust the dose of GLP-1 agonist being administered to a patient with type 1 diabetes, said method comprising:

a) calculating the sum of said patient's HbA_{1c} and four times the patient's daily insulin dose (U/kg/hour) for a first day;

b) calculating the sum of said patient's HbA_{1c} and four times the patient's daily insulin dose (U/kg/hour) for a second day; and

c) comparing the sum obtained in step a) with the sum obtained in step b) where a difference between the sum in step a) and the sum in step b) indicates that the dose of GLP-1 administered to said patient should be adjusted.

Of course, it is understood that since HbA1C is also a biomarker for efficacy of insulin, the above method could also be used to determine whether to adjust the dose of insulin administered to a patient with type 1 diabetes.

In one embodiment, the second day in step b) may be is at least two weeks after the first day in step a).

In another embodiment, the second day in step b) may be is at least four weeks after the first day in step a).

In yet another embodiment, the second day in step b) may be is at least 12 weeks after the first day in step a).

In the above method for determining whether to adjust the dose of GLP-1 agonist, the dose of GLP-1 agonist (and /or insulin) administered to said patient should be increased if the sum in step a) is lower than the sum in step b) and the dose of GLP-1 agonist (and/or insulin) administered to said patient should be decreased if the sum in step a) is greater than the sum in step b).

The present invention further relates to a method for determining whether a patient with type I diabetes is in remission, said method comprising calculating the sum of said patient's HbA_{1c} level on a given day and four times the patient's daily insulin dose for said day, where a sum of less than 9% indicates that said patient is in remission.

In one embodiment, one would repeat the above calculation every 6 weeks, more preferably every 4 weeks, to determine if the patient was in remission.

In another embodiment, the sample to be analyzed is from a patient under 18 years of age.

In yet another embodiment, the sample to be analyzed is from a patient under 16 years of age.

In a further embodiment, the sample to be analyzed is from a patient who is prepubescent.

In yet another embodiment, the sample to be analyzed is from a patient under 12 years of age.

In yet another embodiment, the sample to be analyzed is from a patient under 6 years of age.

In a further embodiment, the above method may be used to determine whether to adjust the dose of GLP-1 agonist being administered to a patient with type 1 diabetes. For

example, if the patient was no longer in remission as determined by the above calculation, one might adjust the dose of GLP-1 agonist. Of course, it is to be understood that treatment with GLP-1 agonist could be continued even if the above calculation indicates that the patient is no longer in remission so long as the patient retained a C peptide level of >100 pmol/l.

Of course, it is understood that since HbA1C is also a biomarker for efficacy of insulin, the above method could also be used to determine whether to adjust the dose of insulin administered to a patient with type 1 diabetes.

All scientific publications and patents cited herein are specifically incorporated by reference. The following examples illustrate various aspects of the invention but are in no way intended to limit the scope thereof.

EXAMPLES

EXAMPLE 1

Relationship Between Levels of endogenous GLP-1 and indicators of beta-cell function during the remission period in children with newly diagnosed type 1 diabetes.

To investigate the relationship between endogenous GLP-1 levels [ie the levels of GLP-1 (7-37) and its metabolite GLP-1 (7-36) amide] and residual beta cell function (as measured by levels of stimulated C-peptide, a surrogate marker for insulin production and hence, beta cell function) in the remission phase of children with type 1 diabetes, the following study was conducted

METHODS

The study was a multicentre longitudinal investigation in 18 paediatric departments representing 15 countries in Europe and Japan. A total number of 276 children and adolescents less than 16 years with newly diagnosed type 1 diabetes presenting to the paediatric departments between August 1999 and December 2000, were eligible for the study. Diabetes remission was defined by the mathematical formula: insulin dose adjusted HbA_{1c}: HbA_{1c} + (4 x the daily insulin dose (U/Kg/24h) < 9%. Thus, a child was said to be in remission if his/her HbA_{1c} + 4 x the daily insulin dose (U/Kg/24h) < 9%.

Exclusion criteria were: suspected non-type-I-diabetes (MODY, secondary diabetes etc.), decline of enrollment into the study by patients or parents and patients initially treated outside of the centres for more than 5 days. Important descriptive data on the 276 patients are presented in Table1.

Table 1: Demographic data by gender

	Female	male	all
number	143	133	276
age	9.6 [0.2 – 16.3]	9.5 [0.7 – 16.8]	9.5 [0.2 – 16.8]
height	135 [74 – 175]	138 [81 – 191]	137 [74 – 191]
weight	28 [9 – 90]	29 [9 – 85]	29 [9 – 90]
BMI	15.5 [10.1–31.1]	16.3 [11.5–33.6]	15.8 [10.1–33.6]
pre-puberty (%)	95 (66.4 %)	101 (75.9 %)	196 (71 %)
menarche occurred	25 (17.5 %)		
White Caucasian (%)	124 (86.7 %)	108 (81.2 %)	232 (84 %)

For each patient, year of birth, sex, duration of symptoms, height, weight, and insulin regimen were recorded. At diagnosis of diabetes, blood glucose, pH, and standard bicarbonate were determined locally by quality-controlled standard laboratory methods. Mild diabetic ketoacidosis (DKA) was defined by the presence of pH < 7.3 and/or a standard bicarbonate of ≤15.0 mmol/l; moderate DKA with a pH limit of <7.2 and/or standard bicarbonate ≤10.0 mmol/l while severe DKA was present if pH was < 7.1 and/or standard bicarbonate ≤5.0 mmol/l.

Samples for HbA_{1c} analysis were collected at onset and after 1, 3, 6, 9 and 12 months after diagnosis of type 1 diabetes at each department using the Bio-Rad HbA_{1c} sample preparation kit (Bio-Rad Laboratories, Munich, Germany) and the HbA_{1c} analysis was performed by automatic high-pressure liquid chromatography at the Steno Diabetes Centre (Denmark) Normal range for HbA_{1c} for the method at Steno Diabetes Center was 4.4 – 6.3 %.

After 1, 6, and 12 month of diabetes, a Boost-challenge (formerly Sustacal) was utilized to stimulate endogenous C-peptide release. The test was performed in the morning after at least 8 hours fasting, the morning insulin dose being given after the test. 6 ml/kg (max: 360 ml) of Boost/Sustacal (Mead Johnson, Evansville, Indiana, USA; 237 ml = 8 FL ounces contains 33 g carbohydrate, 15 g protein and 6 g fat, total of 240 kcal) were ingested in less than 10 minutes. Capillary glucose was measured at time 0 and venous C-peptide, GLP-1(7-37), GLP-1 (7-36) amide, GIP and glucose at 90 minutes after ingestion of sustacal. Serum samples were labelled and frozen at – 20 °C until shipment on dry ice to Steno Diabetes Centre for the determination of C-peptide. Samples were thawed only once for RIA determination. C-peptide was analysed in serum by an enzyme-linked immunosorbent assay (DAKO, Ely, UK)

Samples were analyzed for GLP-1(7-37),GLP-1 (7-36) amide and GIP at the laboratorium, Dept. of Medical Physiol., The Panum Institute, University of Copenhagen.

RESULTS

Generally, endogenous GLP-1 levels increased with time (as C-peptide levels declined in the same period) suggesting an inverse relationship between C-peptide levels (beta-cell function) and GLP-1 over time (see Table 2 and Figures 1-6).

Table 2

	1 mth	n=221	6 mths	n=209	12 mths	n=217
GLP-1 pmol/l	21.7±0.9		22.6±0.9		24.9±0.9	*p<0.001
GIP pmol/l	23.1±1.3		27.0±1.4		28.3±1.3	p<0.01

Values are mean ± SEM

*Repeated measurements model, corrected for age

Specifically, at 1 month (Fig. 1) there is a positive correlation between C-peptide and GLP-1, levels but at 6 months (Fig. 2) and 12 months (Fig. 3) this changes to a negative correlation. By comparison, if HbA1C is used as the surrogate for disease severity (loss of beta-cells), a negative correlation between GLP-1 and HbA1C levels is observed at 1 month (Fig. 4) whereas positive correlations are observed at 3 months (Fig. 5) and 12 months (Fig. 6).

Logistic regression models with C-peptide (beta-cell function) at 1 month, 6 months and 12 months after diagnosis of type 1 diabetes as dependent variables, and 90 min post boost-test GLP-1 and glucose, sex and age as independent variables help to explain this change: at 1 month (Table 3,) GLP-1 is more important than glucose on C-peptide levels, and this explains the positive correlation with C-peptide levels (Fig. 1).

Table 3

Analyses with C-peptide as response at 1 month post-diagnosis

Generally, the logarithmic value is used for C-peptide

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	4.864584870	0.39876486	12.20	<.0001
sex	-0.067969711	0.11820919	-0.57	0.5659
age	0.072660566	0.01607947	4.52	<.0001
loggIp1	0.269301663	0.09520121	2.83	0.0051
bgstim1	-0.031303892	0.01645769	-1.90	0.0586

"log glp" = log endogenous GLP-1 concentration where log glp1 is the log endogenous GLP-1 concentration at 1 month post-diagnosis

"bgstim" = stimulated blood glucose where "bgstim1" is stimulated blood glucose at 1 month post-diagnosis

However, the importance of GLP-1 on C-peptide is lost at 6 months (Table 4) and 12 months (Table 5).

Table 4

Analyses with C-peptide as response at 6 month post-diagnosis

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	5.763267784	0.42198279	13.66	<.0001
sex	0.088756786	0.12653694	0.70	0.4839
age	0.059999635	0.01695082	3.54	0.0005
logglp6	-0.089630342	0.10993399	-0.82	0.4159
bgstim6	-0.042389886	0.01293672	-3.28	0.0012

Table 5

Analyses with C-peptide as response at 12 month post-diagnosis

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	5.929276539	0.53375496	11.11	<.0001
sex	0.003708179	0.14187438	0.03	0.9792
age	0.113323936	0.01953340	5.80	<.0001
logglp12	-0.190065508	0.14641420	-1.30	0.1958
bgstim12	-0.072423686	0.01471627	-4.92	<.0001

Instead, glucose is now the important covariant, and this coincides with negative correlations of C-peptide and GLP-1 at 6 and 12 months (Figures 2 and 3).

If the same models are performed with HbA1C as the dependent variable, the same change occurs between 1 (Fig. 4) month and 3 months (Fig. 5), although HbA1C as a disease marker reflects changes up to 6 weeks earlier. Of interest, GLP-1 at 1 month has significant effects on HbA1C at 3 months and 6 months (Tables 6-8) which is the period in which the highest proportion of the patients are in clinical remission.

Table 6

Analyses with Dose-adjusted HbA1c as response at 3 month post-diagnosis

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	4.159613472	1.03692603	4.01	<.0001
sex	-0.067223008	0.17225515	-0.39	0.6968
age	-0.059424334	0.02621662	-2.27	0.0246
dhba1	0.408825521	0.05259692	7.77	<.0001
lcpep1	-0.149893127	0.10756496	-1.39	0.1651
logglp1	0.547607176	0.14085638	3.89	0.0001

"dhba1" = dose adjusted Hb1Ac at one month post diagnosis

"lcpep1"= log C-peptide concentration at one month post diagnosis

Table 7

Analyses with Dose-adjusted HbA1c as response at 6 month post-diagnosis

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	4.597339656	1.46365041	3.14	0.0020
sex	-0.192356915	0.24109979	-0.80	0.4260
age	-0.028579705	0.03575461	-0.80	0.4252
dhba1	0.403703000	0.07344153	5.50	<.0001
lcpep1	-0.096464423	0.14658293	-0.66	0.5113
logglp1	0.488947027	0.19569765	2.50	0.0134

Table 8

Analyses with Dose-adjusted HbA1c as response at 9 month post-diagnosis

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	5.621778007	1.42854701	3.94	0.0001
sex	-0.248878745	0.24218407	-1.03	0.3056
age	-0.058037648	0.03624229	-1.60	0.1111
dhba1	0.507805788	0.07362262	6.90	<.0001
lcpep1	-0.229057145	0.14446698	-1.59	0.1147
logglp1	0.375243451	0.19947047	1.88	0.0616

GLP-1 as predictor of beta-cell function during remission of type 1 diabetes.

Logistic regression models with C-peptide (beta-cell function) at 6 months, and 12 months as dependent variables and age, sex, C-peptide at 1 month, stimulated blood glucose, and GLP-1 at 1 month were also evaluated (Table 9).

Table 9

Analyses with C-peptide as response at 6 month post-diagnosis

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	2.546098574	0.41636771	6.12	<.0001
sex	0.021162831	0.09509108	0.22	0.8241
age	0.018704375	0.01349071	1.39	0.1673
lcpep1	0.668043536	0.05556939	12.02	<.0001
logglp1	-0.214051281	0.07619402	-2.81	0.0055
bgstim1	-0.043421647	0.01325300	-3.28	0.0013

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	5.792484516	0.42384564	13.67	<.0001
sex	-0.030562330	0.12704284	-0.24	0.8102
age	0.065176394	0.01728568	3.77	0.0002
logglp1	-0.044656114	0.10014287	-0.45	0.6562
bgstim1	-0.059339227	0.01763564	-3.36	0.0009

Analyses with C-peptide as response at 12 month post-diagnosis

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	2.591340287	0.57883960	4.48	<.0001
sex	-0.099930711	0.13011533	-0.77	0.4435
age	0.060818862	0.01852477	3.28	0.0012
lcpep1	0.616683534	0.07677877	8.03	<.0001
logglp1	-0.255919624	0.10504925	-2.44	0.0158
bgstim1	-0.066248485	0.01796387	-3.69	0.0003

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	5.637765989	0.50609587	11.14	<.0001
sex	-0.160659530	0.15034455	-1.07	0.2866
age	0.104812413	0.02048241	5.12	<.0001
logglp1	-0.094489922	0.11934079	-0.79	0.4295
bgstim1	-0.085269745	0.02061041	-4.14	<.0001

The models demonstrate that high endogenous GLP-1 levels at 1 month independently of glucose significantly predict lower C-peptide (and hence, beta-cell function) at 6 and 12 months.

Specifically, when C-peptide at 6 months (log scale) is evaluated in a multiple regression model as a function of sex, age, C-peptide (1 month), stimulated blood glucose (1 month) and GLP1 (1 month-log scale), the coefficient to GLP1 is –0.21 (SE 0.08) ($p<0.01$). For predicting C-peptide at 12 months, the similar coefficient is –0.29 (0.12) ($p<0.02$).

EXAMPLE 2

GLP-1 Intervention Study

One interpretation of the data obtained in Example 1 is that a feed-back mechanism exists in the entero-insular axis, in which the failing beta-cells require more "incretin" effect for survival. However, the GLP-1 secreting L-cells already are maximally stimulated, and therefore, the required increase in GLP-1 is not seen and as the deterioration of beta-cell function continues, the beta cells (especially around 1-6 months) lose their sensitivity to GLP-1 and glucose becomes the most important prandial stimulant of beta-cell function. Pharmacological intervention during this period (remission) with GLP-1 analog is therefore expected to compensate for the required intestinal increase in GLP-1 secretion, and thus further boost/reinforce the survival of residual beta-cell mass, thereby resulting in prolongation of the remission period.

Study Design

A total number of 100 children and adolescents (age below 16 yrs) with newly diagnosed type 1 diabetes will be enrolled to participate in the study. Clinical information on sex, age, pubertal status, duration of symptoms, DKA at presentation, and insulin regimen will be recorded. Baseline C-peptide and glucose will be determined at diagnosis. A stimulated C-peptide test will be carried out in each subject at 1, 6 and 12 months after diagnosis. HbA_{1c} and serum for immunology (ICA, GAD, IA2, IAA) will be analysed with regular intervals in this period. At study entry blood samples are drawn for DNA isolation and HLA typing from each individual.

50 children will be treated with insulin as usual during a period of 1 year and 50 children will be randomised to treatment with the GLP-1 agonists Arg^{34}, $Lys^{26}(N^{\varepsilon}$-(γ-Glu(N^{α}-hexadecanoyl)))-GLP-1(7-37) (Novo Nordisk) or exendin-4 (exanatide from Amylin Inc.) in combination with insulin.

Statistics with power considerations

The statistical evaluation will be based on a repeated measurements model for data on stimulated C-peptide dose-adjusted HbA_{1c} respectively. The analyses will correct for the baseline value. The power has been calculated in a slighter simpler frame, using only the 12-month value as response. This is the final and thus most informative value. It has been calculated how large differences can be obtained with a study size of 100 patients (50 in each group). Values of the variation within and between patients are taken as found in the Hvidøre remission study described in Example 1.

For stimulated C-peptide (logarithmic scale), the variation within patients is 0.39 and between patients 0.59. This implies that the variation on the 12-month value is an SD of 0.62, when the baseline is accounted for. Using a significance level of 0.05 and a power of 0.9, a minimum difference of 0.51 implies that it is necessary to have 50 patients in each group. This difference corresponds to a factor of 1.67 between the two treatment groups, in the stimulated C-peptide after 12 months. This evaluation does not account for dropout.

For dose-adjusted HbA1c (HbA1c % + 4 x daily dose/kg), the variation within patients is 1.74 and between patients 1.92. This implies that the variation on the 12-month value is an SD of 1.63, when the baseline is accounted for. Using a significance level of 0.05 and a power of 0.9, a minimum difference of 1.06 implies that it is necessary to have 50 patients in each group. This evaluation does not account for dropout.

Treatment with a GLP-1 agonist will prolong the remission phase in children with newly diagnosed type 1 diabetes by stimulating the preservation of the patients' residual beta-cell function as measured by a surrogate marker for beta cell function, C peptide levels.

EXAMPLE 3

Relationship between levels of antibodies to glutamic acid decarboxylase (GAD) and indicators of beta-cell function during the remission period in children with newly diagnosed type 1 diabetes.

To investigate the relationship between levels of anti-GAD antibodies and residual beta cell function (as measured by levels of stimulated C-peptide, a surrogate marker for insulin production and hence, beta cell function) in the remission phase of children with type 1 diabetes, the following study was conducted

METHODS

The study was a multicentre longitudinal investigation in 18 paediatric departments representing 15 countries in Europe and Japan. A total number of 276 children and adolescents less than 16 years with newly diagnosed type 1 diabetes presenting to the paediatric departments between August 1999 and December 2000, were eligible for the study. Diabetes remission was defined by the mathematical formula: insulin dose adjusted HbA_{1c}: HbA_{1c} + (4 x the daily insulin dose (U/Kg/24h) < 9%. Thus, a child was said to be in remission if his/her HbA_{1c} + 4 x the daily insulin dose (U/Kg/24h) < 9%.

Exclusion criteria were: suspected non-type-I-diabetes (MODY, secondary diabetes etc.), decline of enrollment into the study by patients or parents and patients initially treated outside of the centres for more than 5 days. Important descriptive data on the 276 patients are presented in Table 10

Table 10: Demographic data by gender

	female	male	all
Number	143	133	276
Age	9.6 [0.2 – 16.3]	9.5 [0.7 – 16.8]	9.5 [0.2 – 16.8]
Height	135 [74 – 175]	138 [81 – 191]	137 [74 – 191]
Weight	28 [9 – 90]	29 [9 – 85]	29 [9 – 90]
BMI	15.5 [10.1–31.1]	16.3 [11.5–33.6]	15.8 [10.1–33.6]
pre-puberty (%)	95 (66.4 %)	101 (75.9 %)	196 (71 %)
menarche occurred	25 (17.5 %)		
White Caucasian (%)	124 (86.7 %)	108 (81.2 %)	232 (84 %)

For each patient, year of birth, sex, duration of symptoms, height, weight, and insulin regimen were recorded. At diagnosis of diabetes, blood glucose, pH, and standard bicarbonate were determined locally by quality-controlled standard laboratory methods. Mild diabetic ketoacidosis (DKA) was defined by the presence of pH < 7.3 and/or a standard bicarbonate of ≤15.0 mmol/l; moderate DKA with a pH limit of <7.2 and/or standard bicarbonate ≤10.0 mmol/l while severe DKA was present if pH was < 7.1 and/or standard bicarbonate ≤5.0 mmol/l.

Samples for HbA_{1c} analysis were collected at onset and after 1, 3, 6, 9 and 12 months after diagnosis of type 1 diabetes at each department using the Bio-Rad HbA_{1c} sample preparation kit (Bio-Rad Laboratories, Munich, Germany) and the HbA_{1c} analysis was performed by automatic high-pressure liquid chromatography at the Steno Diabetes Centre

(Denmark) Normal range for HbA_{1c} for the method at Steno Diabetes Center was 4.4 – 6.3 %.

After 1, 6, and 12 month of diabetes, a Boost-challenge (formerly Sustacal) was utilized to stimulate endogenous C-peptide release. The test was performed in the morning after at least 8 hours fasting, the morning insulin dose being given after the test. 6 ml/kg (max: 360 ml) of Boost/Sustacal (Mead Johnson, Evansville, Indiana, USA; 237 ml = 8 FL ounces contains 33 g carbohydrate, 15 g protein and 6 g fat, total of 240 kcal) were ingested in less than 10 minutes. Capillary glucose was measured at time 0 and venous C-peptide and glucose at 90 minutes after ingestion of sustacal. Serum samples were labelled and frozen at – 20 °C until shipment on dry ice to Steno Diabetes Centre for the determination of C-peptide. Samples were thawed only once for RIA determination. C-peptide was analysed in serum by an enzyme-linked immunosorbent assay (DAKO, Ely, UK)
Samples were quantified for anti-GAD antibodies by direct radio ligand assay at Statens Serum Institut, Dept of Biochemistry, Copenhagen, Denmark.

RESULTS

Anti-GAD antibody titres were significantly associated with age:

Table 11

Relation of age to GADA titres at 12 months

Older children have higher GADA titres

Titres	0-10 RU Percentage (N)	10-100 RU	>=100 RU	Total
Age-group 0-5 years	55.8 (24)	32.3 (14)	11.6 (5)	43
Age-group 5-10 years	39.3 (35)	41.6 (37)	19.1 (17)	89
Age group 10-16 years	32.0 (33)	32.0 (33)	35.9 (37)	103
Total Chi-Square P=0.0048	36	69	77	235

and anti-GAD antibody levels had significant impact on outcome of residual beta-cell function (C-peptide) at 12 months after diagnosis:

Source	DF	Type III SS	Mean Square	F Value	Pr > F
sex	1	0.10368621	0.10368621	0.09	0.7697
age	1	65.16595083	65.16595083	53.99	<.0001
Gadgr12	2	11.25978926	5.62989463	4.66	0.0103

"DF" is degrees of freedom.

"F value" is an F value in an F test.

Furthermore, analyses show that the higher anti-GAD antibody titres the lower residual beta-cell function, thus suggesting that anti-GAD-antibody titres have a direct influence (negative) on disease pathogenesis of type 1 diabetes and the duration of the remission period:

Standard Parameter	Estimate	Error	t Value	Pr > | t |
Intercept	3.492045818 B	0.31875241	10.96	<.0001
Sex	-0.042440114	0.14480423	-0.29	0.7697
Age	0.145742999	0.01983547	7.35	<.0001
Gadgr12 0-10	0.562399543 B	0.19061427	2.95	0.0035
Gadgr12 10-100	0.222346233 B	0.18904656	1.18	0.2408
Gadgr12 >100	0.000000000 B	.	.	.

"gadgr12 0-10"= 0-10 reactive units (RU) of anti-GAD antibody at twelve months post diagnosis

"gadgr12 10-100"= 10-100 reactive units (RU) of anti-GAD antibody at twelve months post diagnosis

"gadgr12 >100"= >100 reactive units (RU) of anti-GAD antibody at twelve months post diagnosis

These data support the potential benefits of intervention in newly onset type 1 diabetes by hyposensibilisation with GAD or GAD-like epitope in newly onset type 1 diabetes in combination with a beta-cell tropic factor for the preservation of residual beta-cell function and a longer remission period.